REPORT

DETERMINATION OF THE HYDROLYSIS OF

[redacted]

AS A FUNCTION OF pH

NOTOX Project 354048
NOTOX Substance 111834/B

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

CONFIDENTIALITY STATEMENT

Study Director

Dr.Ir. E. Baltussen

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Date: 07 NOV 2002

Management

Dr. Ir. H. Willems
Section Head
Analytical & Physical Chemistry

....

Date: November 07, 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection (s)	
13 – 31 May 2002 (Process, physical chemistry)	04 June 2002
protocol inspection (s)	
31 May 2002 (Study)	31 May 2002
report audit (s)	
17 – 18 October 2002 (Study)	18 October 2002

Head of Quality Assurance
C.J. Mitchell B.Sc.
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.... J.T.G. Wiltschut

Date: November 05, 2002

SUMMARY

The determination of the hydrolysis rate of [redacted] as a function of pH was based on the EEC-Directive 92/69 EEC, Part C, Methods for the determination of Ecotoxicity, C.7: "Abiotic degradation: Hydrolysis as a function of pH", EEC Publication no. L383, December 1992.

According to information supplied by the sponsor, [redacted] is a formulation containing several components (see the certificate of analysis). Measurements were performed on the following components: MIPK, MIPKP-T4 and MIPKP-T3 (peak 1 and peak 2). Due to the fact that DMP, which was used for preparation of this formulation, is a very well known compound, which cannot be determined in the same chromatographic run as the other compounds due to a large difference in sensitivity (see also Notox Project 338805 "Development and validation of an analytical method for [redacted]), analyses were not based on this compound. Due to an interfering peak in blank buffer solutions at the retention time of Hydrogen peroxide it was not possible to determine the hydrolysis of this component. Hydrogen peroxide is a very well known compound and therefore this was not considered to have an effect on this study.

MIPK is a degradation product of the other compounds present in the formulation. Therefore it was not possible to determine the hydrolysis of this component.

MIPKP-T4 is hydrolytically stable (half-life time at 25°C > 1 year) in aqueous solutions buffered at pH 9.

The half-life time for MIPKP-T4 at 25°C is 411 and 7943 hours in aqueous solutions buffered at pH 4 and pH 7 respectively.

The half-life time for MIPKP-T3 peak 1 at 25°C is 145, 1438 and 147 hours in aqueous solutions buffered at pH 4, pH 7 and pH 9 respectively.

The half-life time for MIPKP-T3 peak 2 at 25°C is 98.8, 1221 and 61.1 hours in aqueous solutions buffered at pH 4, pH 7 and pH 9 respectively.

PREFACE

Sponsor	[redacted]
Study Monitor	Dr. C.L.J. Braun SHERA, Regulatory Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Dr. Ir. E. Baltussen
Study plan	Start: 26 June 2002 Completed: 16 August 2002

TEST SUBSTANCE

[redacted]

PURPOSE AND PRINCIPLE

The purpose of the study was to examine hydrolysis of the test substance at pH values normally found in the environment (pH 4-9).

The test substance was dissolved in aqueous solutions buffered at specific pH values (pH 4, pH 7 and pH 9) and incubated at constant temperature(s). The concentration of the test substance was determined as a function of time.

GUIDELINES

The study procedure described in this report was based on the following guideline:

European Economic Community (EEC), EEC-Directive 92/69 EEC, Part C, Methods for the determination of Ecotoxicity, C.7: "Abiotic degradation: Hydrolysis as a function of pH", EEC Publication no. L383, December 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:
protocol, report, test substance reference sample and raw data. Thereafter, no data will be withdrawn without the sponsor's written consent.

TEST SYSTEM AND RATIONALE

A sterile 0.05 M acetate buffer pH 4: sodium acetate/acetic acid/Milli-Q water.

A sterile 0.05 M phosphate buffer pH 7: potassium dihydrogen phosphate/ sodium hydroxide/Milli-Q water.

A sterile 0.05 M borate buffer pH 9: boric acid/potassium chloride/sodium hydroxide/Milli-Q water.

Each test system is recognized by the international guideline (EEC).

VALIDATION OF THE TEST PROCEDURE

The test method as outlined in this report is validated periodically, using acetylsalicylic acid. The results were in accordance with the NOTOX criteria for the validation.

REAGENTS

Sodium acetate	P.a., Merck, Darmstadt, Germany
Acetic acid	100%, p.a., Merck
Potassium dihydrogen phosphate	P.a., Merck
Sodium hydroxide, 1 M	Merck
Boric acid	P.a., Merck
Potassium chloride	P.a., Merck
Acetonitrile	HPLC-grade, Labscan, Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore, Bedford, MA, USA

PERFORMANCE OF THE TEST

Preparation of the test solutions

Prior to the start of each test, the test solutions were freshly prepared. For the preparation of the test solutions, 50 ml of each buffer solution (pH 4, pH 7 or pH 9) was filter-sterilised through a 0.2 μm membrane filter (FP 30/0.2 CA-S, Schleicher & Schuell, Dassel, Germany) and transferred into sterile glass vessels. To exclude oxygen, nitrogen gas was bubbled through each solution for 5 minutes. Then, 500 μl of a 23.6 g/l solution of [redacted] in acetonitrile was added to each of the sterile buffer solutions. Thereafter, each vessel was tightly sealed with a septum-crimpcap.

Preliminary test according to EEC Guideline C.7.

The test solutions at pH 4, pH 7 and pH 9 were placed in a thermostatically controlled waterbath at 50.0 ± 0.5°C in the dark. The concentration of the test substance was determined immediately after preparation (t=0), after 2.4 hours and after 5 days of incubation. At each sampling point, a 2.5 ml sample was taken for pretreatment and analysis.

Subsequent tests (Tests 1 and 3 according to EEC Guideline C.7.)

During the preliminary test, significant hydrolysis (i.e. a decrease in test substance concentration at 50°C < 50% after 2.4 hours but > 10% after 5 days) was observed at pH 4, pH 7 and pH 9 for MIPKP-T3. Therefore, subsequent tests were carried out at pH 4 (at 50.0 ± 0.5°C and at 39.0 ± 0.5°C), at pH 7 (at 60.0 ± 0.5°C and at 70.0 ± 0.5°C) and at pH 9 (at 50.0 ± 0.5°C and at 39.0 ± 0.5°C).

During the preliminary test, significant hydrolysis (i.e. a decrease in test substance concentration at 50°C < 50% after 2.4 hours but > 10% after 5 days) was observed at pH 4 and pH 7 for MIPKP-T4. Therefore, subsequent tests were carried out at pH 4 (at 50.0 ± 0.5°C and at 70.0 ± 0.5°C) and at pH 7 (at 80.0 ± 0.5°C and at 90.0 ± 0.5°C).

After preparation, each test solution was placed in a thermostatically controlled waterbath at the specified temperature and in the dark. The concentration of the test substance in each solution was determined immediately after preparation (t=0) and several sampling points after t=0. At each sampling point, a 1 – 2.5 ml sample was taken for pretreatment and analysis.

Testing of pseudo-first order kinetics 1 (Test 1 according to EEC Guideline C.7)

For each test solution, the logarithms of the relative concentrations between 80% and 30% (i.e. between 20% and 70% hydrolysis) were plotted against time.

Determination of the half-life time at 25°C (Test 3 according to EEC guideline C.7)

Because the lines from Test 1 were straight lines, Test 3 was carried out. Therefore, the logarithms of all relative concentrations were plotted against time (for each test solution). The half-life times for MIPKP-T3 at 50°C and 39°C (for pH 4), 60°C and 70°C (for pH 7) or at 50°C and 39°C (for pH 9) were calculated from the resultant lines. The half-life times for MIPKP-T4 at 50°C and 70°C (for pH 4) or at 80°C and 90°C (for pH 7) were also calculated from the resultant lines. Using these values, the half-life time at 25°C was calculated.

pH measurements

For each test solution, the pH value at room temperature was determined at the beginning and at the end of each test.

METHOD OF CHEMICAL ANALYSIS

The concentration of [redacted] was determined using a High Performance Liquid Chromatographic method. The conditions used are described below.

According to information supplied by the sponsor, [redacted] is a formulation containing several components (see the certificate of analysis). Measurements were performed on the following components: MIPK, MIPKP-T4 and MIPKP-T3 (peak 1 and peak 2). Due to the fact that DMP, which was used for preparation of this formulation, is a very well known compound, which cannot be determined in the same chromatographic run as the other compounds due to a large difference in sensitivity (see also Notox Project 338805 "Development and validation of an analytical method for Trigonox R-938"), analyses were not based on this compound. Due to an interfering peak in blank buffer solutions at the retention time of Hydrogen peroxide it was not possible to determine the hydrolysis of this component. Hydrogen peroxide is a very well known compound and therefore this was not considered to have an effect on this study.

A typical HPLC chromatogram of [redacted] is shown in Figure 1.

MIPK is a degradation product of the other compounds present in the formulation. Therefore it was not possible to determine the hydrolysis of this component.

Sample pretreatment

The samples taken at t>0 were cooled to room temperature by running tap-water immediately after sampling. Thereafter, each sample was diluted with acetonitrile in a 1:1 ratio to obtain concentrations within the calibration range and analysed.

Blank solutions

During the preliminary test:

On the first day of each test, blank buffer solutions were diluted with acetonitrile with the same factor as the corresponding test solutions.

During the main study:

On the first day of each test, blank buffer solutions containing between 0.55 and 0.70% (v/v) acetonitrile were diluted with acetonitrile with the same factor as the corresponding test solutions.

Analytical method

Column	Zorbax RX-C18, 250 x 4.6 mm; d_p=5 μm (Chrompack, Middelburg, the Netherlands)
Mobile phase A	Acetonitrile
Mobile phase B	Milli-Q water
Gradient program	

Time (min.)	%A	%B
0	46	54
5	46	54
10	100	0
13	100	0
14	46	54
19	46	54

Flow	2 ml/min
Detection wavelength	260 nm (for MIPK) and 220 nm (for all other components)
Injection volume	100 μl

Standard and calibration solutions

Standard solutions of [redacted] were prepared in acetonitrile.

On each day of analysis, calibration solutions in 50/50 (v/v) acetonitrile/0.05 M acetate buffer pH 4, 50/50 (v/v) acetonitrile/0.05 M phosphate buffer pH 7 and 50/50 (v/v) acetonitrile/0.05 M borate buffer pH 9 were made up from two standard solutions.

DATA HANDLING

General

Mean:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

where
x_i = measured value
n = number of measurements

Maximum deviation: [(highest value - lowest value)/mean] * 100%

Calibration

Response: R = Peak area test substance [units]

Calibration curve: The response of the calibration solution was correlated with the test substance concentration, using linear regression analysis (least squares method; when necessary a weighting factor (1/concentration) was used).

$$R = a * C + b$$

R = response calibration solution [units]
C = concentration of test substance in calibration solution [mg/l]
a = slope [units*l/mg]
b = intercept [units]

Calibration curves were constructed using at least six concentrations. For each concentration, two responses were used. The coefficient of correlation was always >0.997.

Samples

Concentration of [redacted] in the samples:

$$C = \frac{(R-b) * d}{a} \quad [\text{mg/l}]$$

R = response sample [units]
d = dilution factor
a = slope of the calibration curve [units*l/mg]
b = intercept of the calibration curve [units]

Hydrolysis tests

The decrease in concentration (preliminary test) or the degree of hydrolysis (subsequent tests) was calculated using the formula:

$$[(C_0 - C_t)/C_0]*100\%$$

where
C_0 = concentration at time 0
C_t = concentration at time t

Relative concentration: $C_r = [C_t/C_0]*100\%$

Half-life time: $t_{½}$ = the time required to reduce the concentration of the test substance by 50%.

For the testing of pseudo-first order kinetics (Test 1 according to EEC guideline C.7) and for the determination of the half-life time ($t_{½}$) (Test 3 according to EEC guideline C.7), ${}^{10}\log C_r$ (y-value) was plotted against time (x-value).

The testing of pseudo-first order kinetics
The logarithms of the relative concentrations between 80% and 30% (i.e. between 20% and 70% hydrolysis) were plotted against the sampling points.

Determination of the half-life time ($t_{½}$) at 25°C

The logarithms of all relative concentrations were plotted against the sampling points. A linear regression program (least squares method) was used to calculate the regression line. The rate constant, k_{obs}, was calculated using the following formula:

k_{obs} = -slope * 2.303 , where the slope is from the regression line

The half-life time ($t_{½}$) of the reaction was calculated by: $t_{½} = 0.693/k_{obs}$

At each pH value at which the rate constants at two different temperatures are known, the rate constant (and the half-life time) at 25°C was estimated by applying the Arrhenius equation:

$\ln k_{obs} = c1 - c2 * 1/T$

where T = temperature (in K) and c1, c2 = constant values

RESULTS

All relative concentrations and logarithms therefrom were calculated using not-rounded concentrations. Therefore, some differences might be observed when re-calculating the relative concentrations and logarithms therefrom using the concentrations as mentioned in the tables below.

Preliminary test

The results of the preliminary test are summarised in Tables 1 to 3. No test substance was measured in the blank buffer solutions.

Table 1 Results of the preliminary test at 50.0 ± 0.5°C for MIPKP-T4.

pH code	Sampling time	Concentration of the test substance based on MIPKP-T4 [mg/l] [1]	Relative concentration [%]	Measured pH value
4	0 hours	242	100	4.0
	2.4 hours	222	91.6	4.0
	5 days	39.1 [2]	16.2	4.1
7	0 hours	226	100	7.1
	2.4 hours	234	104	7.1
	5 days	153	67.7	7.1
9	0 hours	245	100	9.1
	2.4 hours	275	112	9.0
	5 days	273	111	9.0

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%.
2 Calculated by extrapolation of the calibration curve.

Table 2 Results of the preliminary test at 50.0 ± 0.5°C for MIPK.

pH code	Sampling time	Concentration of the test substance based on MIPK [mg/l] [1,2]	Relative concentration [%]	Measured pH value
4	0 hours	258	100	4.0
	2.4 hours	646 [3]	251	4.0
	5 days	3685 [3]	1429	4.1
7	0 hours	242	100	7.1
	2.4 hours	266	110	7.1
	5 days	1289 [3]	532	7.1
9	0 hours	285	100	9.1
	2.4 hours	454 [3]	160	9.0
	5 days	911 [3]	320	9.0

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%.
2 The calibration lines for MIPK were very pore therefore the results are not reliable.
3 Calculated by extrapolation of the calibration curve.

Table 3 Results of the preliminary test at 50.0 ± 0.5°C for MIPKP-T3.

pH code	Sampling time	Peak	Concentration of the test substance based on MIPKP-T3 [mg/l] [1]	Relative concentration [%]	Measured pH value
4	0 hours	1	239	100	4.0
		2	234	100	
	2.4 hours	1	202	84.7	4.0
		2	184	78.6	
	5 days	1	n.d.	0	4.1
		2	n.d.	0	
7	0 hours	1	227	100	7.1
		2	221	100	
	2.4 hours	1	228	100	7.1
		2	220	99.6	
	5 days	1	28.8 [2]	12.7	7.1
		2	16.8 [2]	7.6	
9	0 hours	1	226	100	9.1
		2	252	100	
	2.4 hours	1	157	69.4	9.0
		2	148	58.9	
	5 days	1	n.d.	0	9.0
		2	n.d.	0	

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%.
2 Calculated by extrapolation of the calibration curve.
n.d. No test substance was detected in the test solution

MIPK is a degradation product of the other compounds present in the formulation. Therefore it was not possible to determine the hydrolysis of this component.

At pH 9 for MIPKP-T4, a decrease in concentration < 10% was observed after 5 days (half-life time at 25°C > 1 year). Therefore, no further testing was necessary at pH 9 for MIPKP-T4.

At pH 4 (for MIPKP-T3 and MIPKP-T4), pH 7 (for MIPKP-T3 and MIPKP-T4) and pH 9 (for MIPKP-T3), a decrease in concentration < 50% after 2.4 hours but > 10% after 5 days was observed. In order to determine if hydrolysis of TRIGONOX R-938 is a pseudo-first order reaction and to determine the half-life time at 25°C, subsequent tests were performed at these pH values and for these compounds.

Subsequent tests

The analytical results of the subsequent hydrolysis tests are summarised in Tables 4 to 6. No test substance was measured in the blank buffer solutions.

Table 4 Results of hydrolysis tests at pH 4 and pH 7 for MIPKP-T4.

Code pH and temperature	Measured pH value	Time [hours]	Concentration for the test substance based on MIPKP-T4 [mg/l] [1]	Relative concentration [%]	Logarithm relative Concentration [2]
pH 4 - 50°C	4.0	0	236	100	2.00
		2	206	87.5	1.94
		3	199	84.5	1.93
		4	188	79.9	1.90 [3]
		5	175	74.4	1.87 [3]
		6	172	72.8	1.86 [3]
	4.0	7	164	69.5	1.84 [3]
pH 4 – 70°C	4.0	0	199	100	2.00
		2	84.6	42.6	1.63
		4	29.5 [4]	14.8	1.17
		5	16.0 [4]	8.07	0.91
		6	7.41 [4]	3.73	0.57
	4.0	7	4.21 [4]	2.12	0.33
pH 7 - 80°C	7.0	0	214	100	2.00
		0.5	213	99.6	2.00
		1.0	208	97.0	1.99
		1.5	191	89.3	1.95
		2.0	182	85.0	1.93
		2.5	172	80.5	1.91
	7.1	3.0	167	77.8	1.89 [3]
	7.1	4.0	149	69.6	1.84 [3]
	7.0	5.0	135	63.2	1.80 [3]
pH 7 - 90°C	7.0	0	224	100	2.00
		0.5	200	98.1	1.95
		1.0	175	78.0	1.89
		1.5	151	67.6	1.83
		2.0	132	58.9	1.77
		2.5	114	50.9	1.71
	7.1	3.0	98.8	44.2	1.65
	7.0	4.0	73.9	33.0	1.52
	7.0	5.0	56.8	25.4	1.40

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%
2 Values used for the determination of the half-life times.
3 Value between 20% and 70% hydrolysis. Used for the testing of (pseudo-) first order kinetics.
4 Calculated by extrapolation of the calibration curve.

Table 5 Results of hydrolysis tests at pH 4, pH 7 and pH 9 for MIPKP-T3 peak 1.

Code pH and temperature	Measured pH value	Time [hours]	Concentration test of the substance based on MIPKP-T3 peak 1 [mg/l] [1]	Relative concentration [%]	Logarithm relative Concentration [2]
pH 4 – 50°C	4.0	0	216	100	2.00
		2	175	81.3	1.91
		3	164	75.8	1.88 [3]
		4	146	67.9	1.83 [3]
		5	137	63.7	1.80 [3]
		6	126	58.4	1.77 [3]
	4.0	7	117	54.1	1.73 [3]
pH 4 – 39°C	3.9	0	221	100	2.00
		17	141	63.8	1.80
		18	139	62.9	1.80
	4.0	19	136	61.7	1.79
	4.0	23	122	55.4	1.74
pH 7 – 60°C	7.1	0	206	100	2.00
		2.0	169	81.9	1.91
		3.0	160	77.6	1.89 [3]
		4.0	146	70.9	1.85 [3]
		5.0	140	67.9	1.83 [3]
		5.5	135	65.4	1.82 [3]
		6.0	131	63.4	1.80 [3]
	7.1	7.0	124	60.2	1.78 [3]
pH 7 – 70°C	7.1	0	201	100	2.00
		2.0	116	57.9	1.76
		4.0	71.3	35.5	1.55
		5.0	56.2	27.9	1.45
		6.0	45.6	22.7	1.36
	7.2	7.0	35.7 [4]	17.8	1.25
pH 9 – 50°C	9.0	0	224	100	2.0
		1.0	199	88.6	1.95
		2.0	170	75.7	1.88 [3]
		3.0	141	62.9	1.80 [3]
		4.0	117	52.2	1.72 [3]
		5.0	92.6	41.3	1.62 [3]
	9.0	6.0	73.5	32.8	1.52 [3]
	9.0	7.0	63.0	28.1	1.45

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%
2 Values used for the determination of the half-life times.
3 Value between 20% and 70% hydrolysis. Used for the testing of (pseudo-) first order kinetics.
4 Calculated by extrapolation of the calibration curve.

Table 5 Continued

Code pH and temperature	Measured pH value	Time [hours]	Concentration of the test substance based on MIPKP-T3 peak 1 [mg/l] [1]	Relative concentration [%]	Logarithm relative Concentration [2]
pH 9 – 39°C	9.1	0.0	211	100	2.00
		17.0	111	52.8	1.72
		18.0	103	49.0	1.69
		20.0	93.5	44.4	1.65
	9.1	22.0	87.8	41.6	1.62
	9.1	24.0	80.8	38.3	1.58

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%
2 Values used for the determination of the half-life times.

Table 6 Results of hydrolysis tests at pH 4, pH 7 and pH 9 for MIPKP-T3 peak 2.

Code pH and temperature	Measured pH value	Time [hours]	Concentration of the test substance based on MIPKP-T3 peak 2 [mg/l] [1]	Relative concentration [%]	Logarithm relative Concentration [2]
pH 4 – 50°C	4.0	0	215	100	2.00
		2	154	71.8	1.86 [3]
		3	141	65.9	1.82 [3]
		4	120	56.1	1.75 [3]
		5	109	50.9	1.71 [3]
		6	98.5	45.9	1.66 [3]
	4.0	7	89.1	41.5	1.62 [3]
pH 4 – 39°C	3.9	0	217	100	2.00
		17	114	52.6	1.72
		18	109	50.0	1.70
	4.0	19	110	50.7	1.71
	4.0	23	92.6	42.7	1.63
pH 7 – 60°C	7.1	0	198	100	2.00
		2.0	155	78.1	1.89 [3]
		3.0	146	73.6	1.87 [3]
		4.0	135	68.1	1.83 [3]
		5.0	127	64.2	1.81 [3]
		5.5	119	60.2	1.78 [3]
		6.0	116	58.6	1.77 [3]
	7.1	7.0	109	54.9	1.74 [3]

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%
2 Values used for the determination of the half-life times.
3 Value between 20% and 70% hydrolysis. Used for the testing of (pseudo-) first order kinetics.

Table 6 Continued

Code pH and temperature	Measured pH value	Time [hours]	Concentration of the test substance based on MIPKP-T3 peak 2 [mg/l] [1]	Relative concentration [%]	Logarithm relative Concentration [2]
pH 7 – 70°C	7.1	0	199	100	2.00
		2.0	103	51.8	1.71
		4.0	58.1	29.2	1.47
		5.0	46.5	23.3	1.37
		6.0	34.5 [4]	17.3	1.24
	7.2	7.0	26.5 [4]	13.3	1.12
pH 9 – 50°C	9.0	0	227	100	2.00
		1.0	177	78.2	1.89 [3]
		2.0	140	61.8	1.79 [3]
		3.0	112	49.4	1.69 [3]
		4.0	83.3	36.7	1.56 [3]
		5.0	65.7	29.0	1.46
	9.0	6.0	51.5	22.7	1.36
	9.0	7.0	40.9 [4]	18.0	1.26
pH 9 – 39°C	9.0	0	228	100	2.00
		3.0	193	84.8	1.93
		4.0	174	76.4	1.88
		5.0	165	72.5	1.86
		6.0	154	67.5	1.83
	9.0	7.0	142	62.4	1.80

1 Mean of duplicate analysis. The maximum deviation between the responses was < 10%
2 Values used for the determination of the half-life times.
3 Value between 20% and 70% hydrolysis. Used for the testing of (pseudo-) first order kinetics.
4 Calculated by extrapolation of the calibration curve.

Testing of pseudo-first order kinetics (Test 1 according to EEC guideline C.7)

For the test solutions at pH 4 (for MIPKP-T3 and MIPKP-T4), pH 7 (for MIPKP-T3 and MIPKP-T4) and pH 9 (for MIPKP-T3), the plots of the logarithms of the relative concentrations between 80% and 30% (i.e. between 20% and 70% hydrolysis) against time were straight lines. Therefore, the reactions at pH 4, pH 7 and pH 9 are considered to be (pseudo)-first order. Hence, the half-life times at 25°C were estimated in a subsequent test.

Determination of the half-life time (t½) at 25°C (Test 3 according to EEC guideline C.7)

The logarithms of all relative concentrations were plotted against time and a linear regression program (least-squares method) was used to calculate the regression line. The equations of the regression lines are summarised in Tables 7 and 8. Figures 2 to 17 show all the regression lines.

Table 7 Regression lines (logarithms of all relative concentrations against time) for MIPKP-T4.

pH	Temperature [°C]	Regression line
4	50	Y = -0.0224X + 1.99
	70	Y = -0.242X + 2.07
7	80	Y = -0.0424X + 2.02
	90	Y = -0.121X + 2.01

Table 8 Regression lines (logarithms of all relative concentrations against time) for MIPKP-T3.

pH	Peak	Temperature [°C]	Regression line
4	1	50	Y = -0.0378X + 1.99
	1	39	Y = -0.0112X + 2.00
	2	50	Y = -0.0537X + 1.98
	2	39	Y = -0.0161X + 2.00
7	1	60	Y = -0.0308X + 1.99
	1	70	Y = -0.106X + 1.99
	2	60	Y = -0.0359X + 1.98
	2	70	Y = -0.124X + 1.98
9	1	50	Y = -0.0821X + 2.03
	1	39	Y = -0.0174X + 2.00
	2	50	Y = -0.107X + 2.00
	2	39	Y = -0.0294X + 2.01

From each regression line, the rate constant and the corresponding half-life time were calculated. Also, the rate constant (and the corresponding half-life time) at 25°C was calculated using the Arrhenius equation. The results are summarised in Tables 9 and 10.

Table 9 Rate constants and half-life times for MIPKP-T4.

pH	Temperature [°C]	Rate constant k_{obs} [hours^{-1}]	Half-life time $t_{1/2}$ [hours]
4	50	0.0517	13.4
4	70	0.558	1.24
4	25	0.00169	411 #
7	80	0.0977	7.09
7	90	0.279	2.49
7	25	0.0000872	7943 #

Estimated using the Arrhenius equation.

Table 10 Rate constants and half-life times for MIPKP-T3.

pH	Peak	Temperature [°C]	Rate constant k_{obs} [hours^{-1}]	Half-life time $t_{1/2}$ [hours]
4	1	50	0.0870	7.96
4	1	39	0.0257	27.0
4	1	25	0.00478	145 #
4	2	50	0.124	5.60
4	2	39	0.0370	18.7
4	2	25	0.00702	98.8 #
7	1	60	0.0710	9.76
7	1	70	0.0245	2.83
7	1	25	0.000482	1438 #
7	2	60	0.0828	8.37
7	2	70	0.285	2.43
7	2	25	0.000568	1221 #
9	1	50	0.189	3.67
9	1	39	0.0401	17.3
9	1	25	0.00471	147 #
9	2	50	0.247	2.81
9	2	39	0.0676	10.2
9	2	25	0.0113	61.1 #

Estimated using the Arrhenius equation.

CONCLUSION

MIPKP-T4 is hydrolytically stable (half-life time at 25°C > 1 year) in aqueous solutions buffered at pH 9.

The half-life time for MIPKP-T4 at 25°C is 411 and 7943 hours in aqueous solutions buffered at pH 4 and pH 7 respectively.

The half-life time for MIPKP-T3 peak 1 at 25°C is 145, 1438 and 147 hours in aqueous solutions buffered at pH 4, pH 7 and pH 9 respectively.

The half-life time for MIPKP-T3 peak 2 at 25°C is 98.8, 1221 and 61.1 hours in aqueous solutions buffered at pH 4, pH 7 and pH 9 respectively.

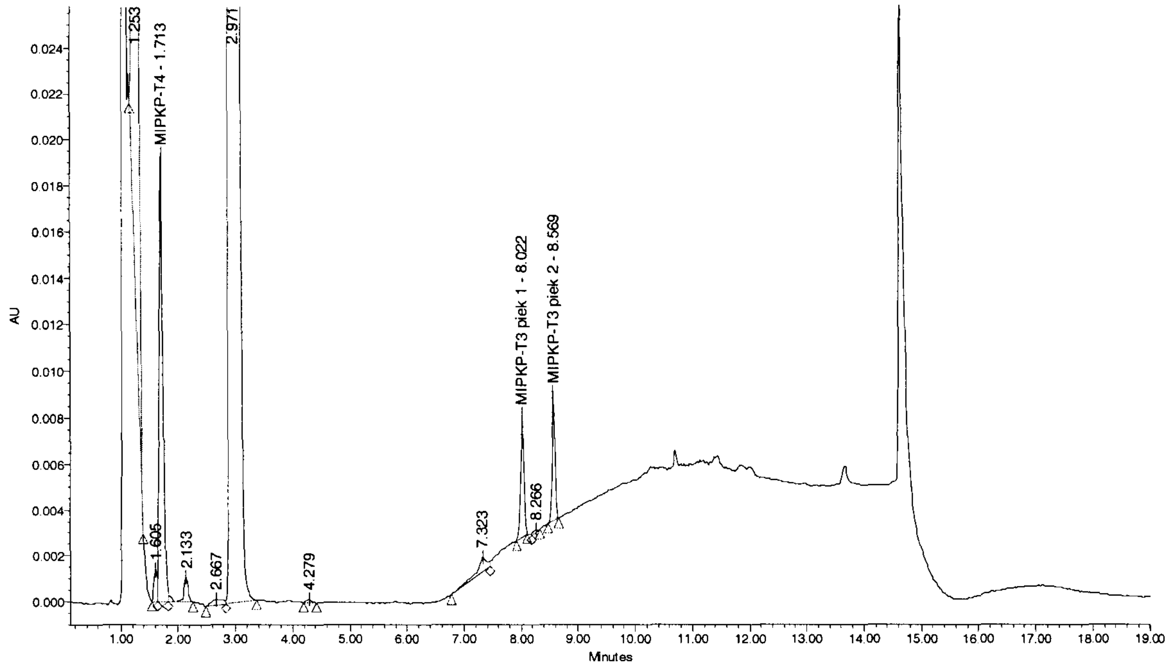

Figure 1 HPLC chromatogram of 200 mg/l test substance solution in 50/50 (v/v) Methanol/0.05 M acetate buffer pH 4 [result id 3564]

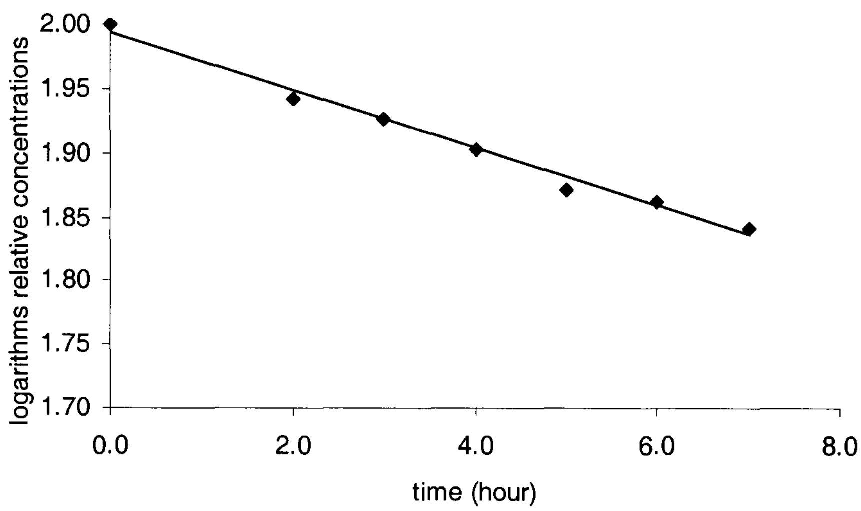

Figure 2 Plot of the logarithms of all relative concentrations against time for pH 4 at 50°C for MIPKP-T4

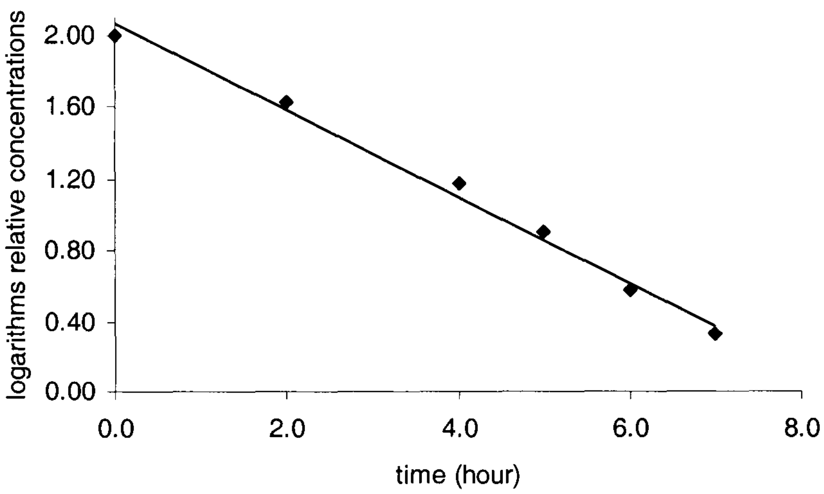

Figure 3 Plot of the logarithms of all relative concentrations against time for pH 4 at 70°C for MIPKP-T4.

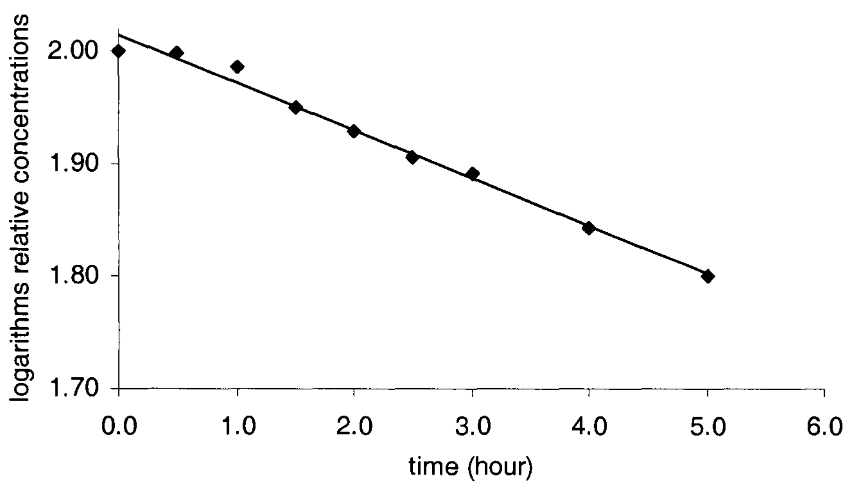

Figure 4 Plot of the logarithms of all relative concentrations against time for pH 7 at 80°C for MIPKP-T4.

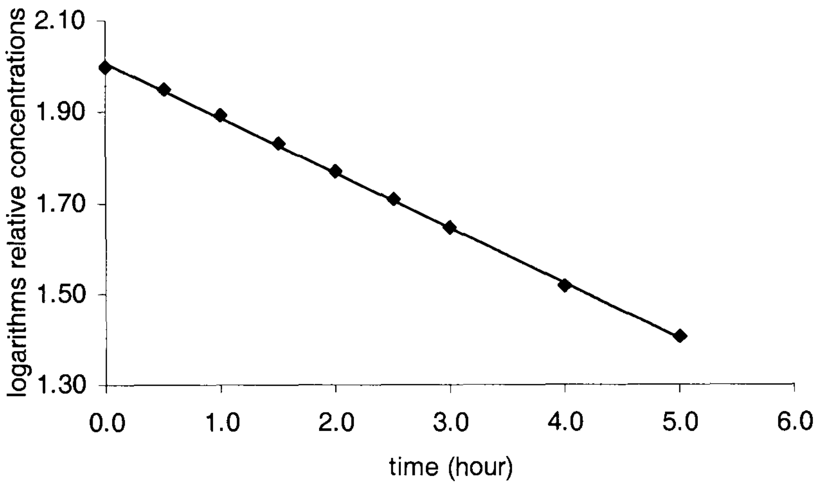

Figure 5 Plot of the logarithms of all relative concentrations against time for pH 7 at 90°C for MIPKP-T4.

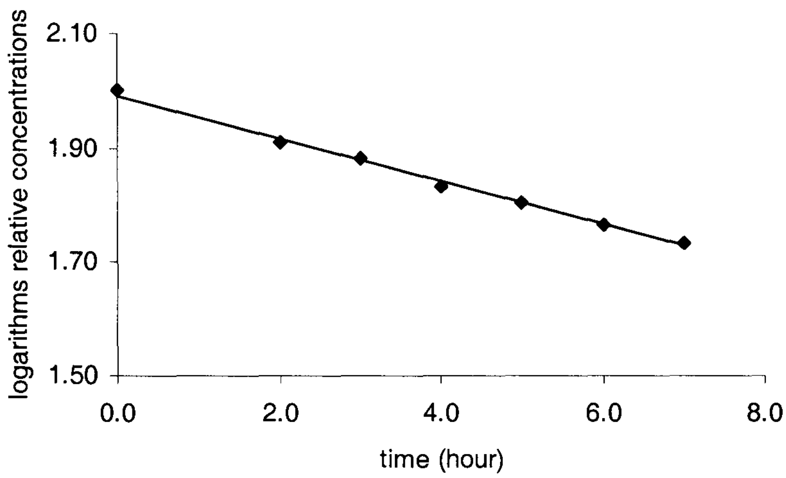

Figure 6 Plot of the logarithms of all relative concentrations against time for pH 4 at 50°C for MIPKP-T3 based on peak 1.

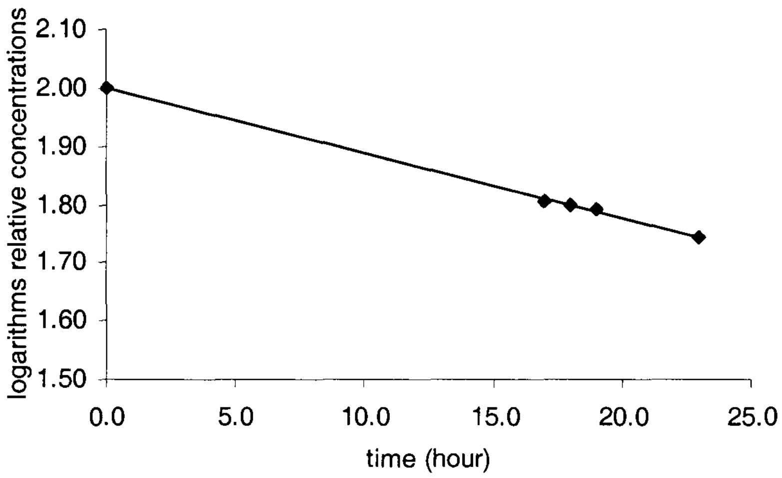

Figure 7 Plot of the logarithms of all relative concentrations against time for pH 4 at 39°C for MIPKP-T3 based on peak 1.

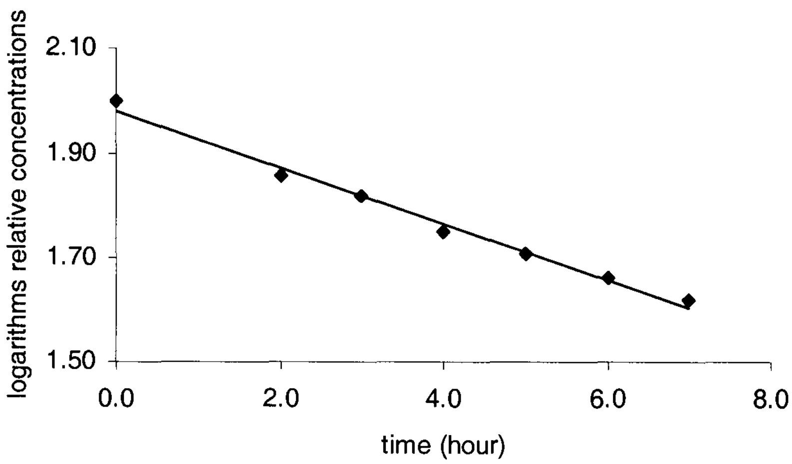

Figure 8 Plot of the logarithms of all relative concentrations against time for pH 4 at 50°C for MIPKP-T3 based on peak 2.

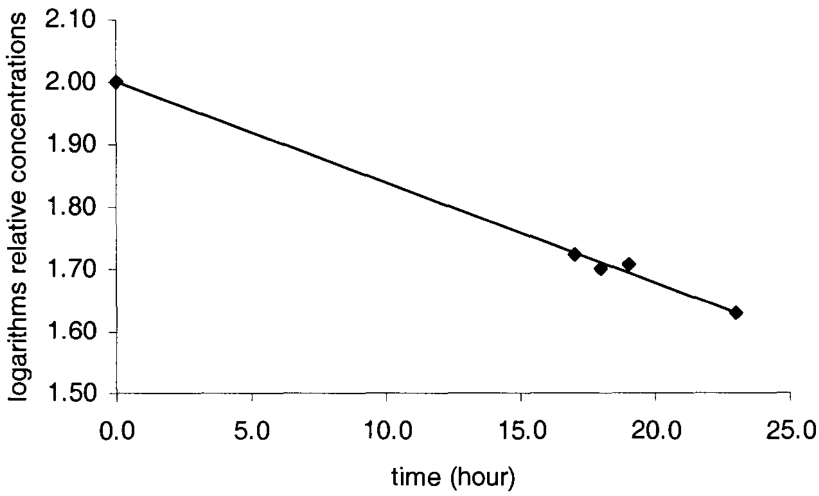

Figure 9 Plot of the logarithms of all relative concentrations against time for pH 4 at 39°C for MIPKP-T3 based on peak 2.

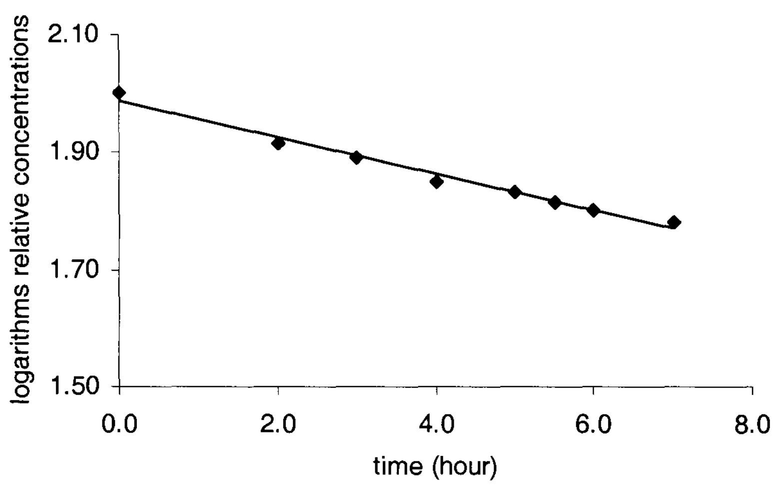

Figure 10 Plot of the logarithms of all relative concentrations against time for pH 7 at 60°C for MIPKP-T3 based on peak 1.

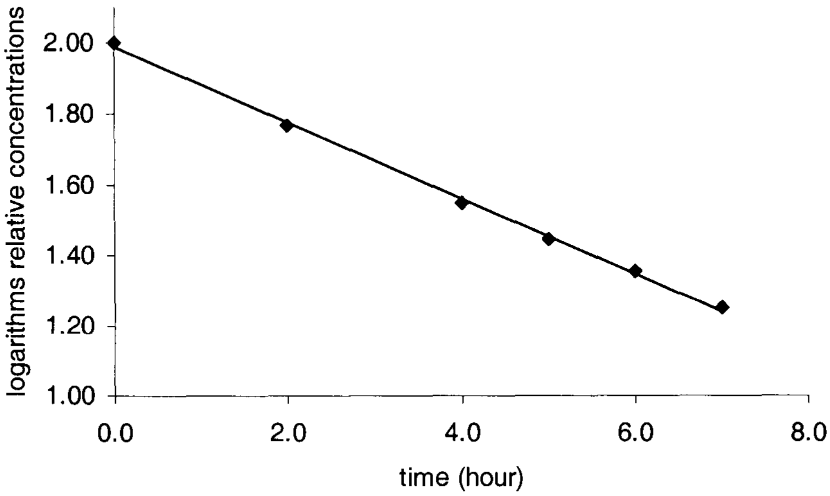

Figure 11 Plot of the logarithms of all relative concentrations against time for pH 7 at 70°C for MIPKP-T3 based on peak 1.

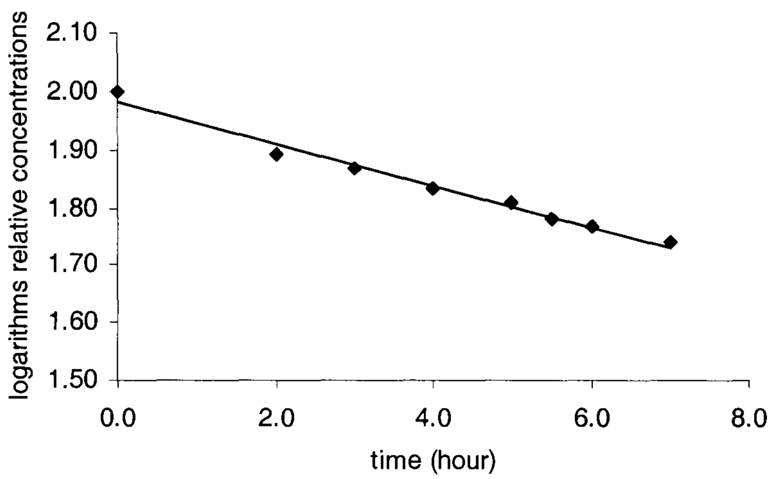

Figure 12 Plot of the logarithms of all relative concentrations against time for pH 7 at 60°C for MIPKP-T3 based on peak 2.

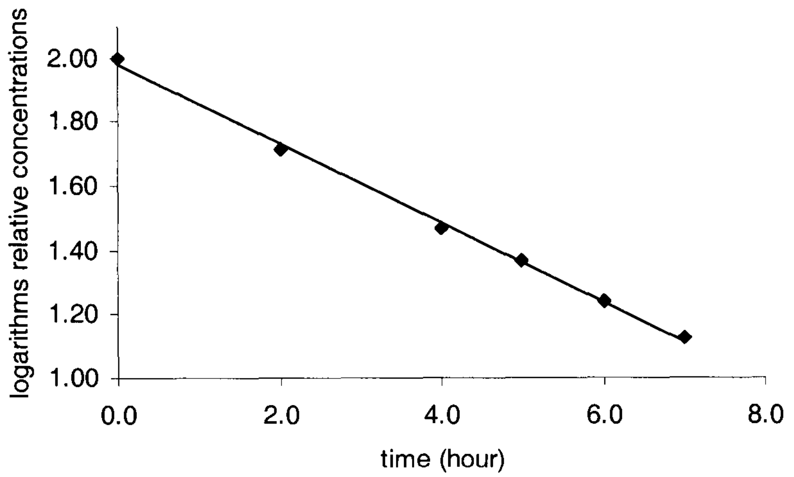

Figure 13 Plot of the logarithms of all relative concentrations against time for pH 7 at 70°C for MIPKP-T3 based on peak 2.

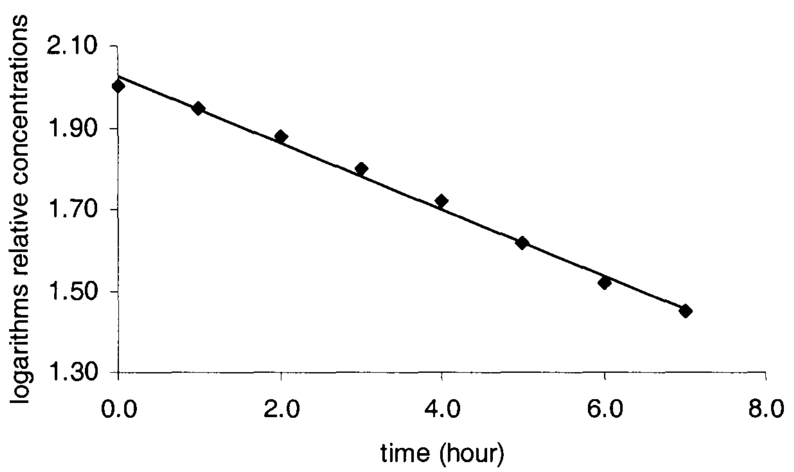

Figure 14 Plot of the logarithms of all relative concentrations against time for pH 9 at 50°C for MIPKP-T3 based on peak 1.

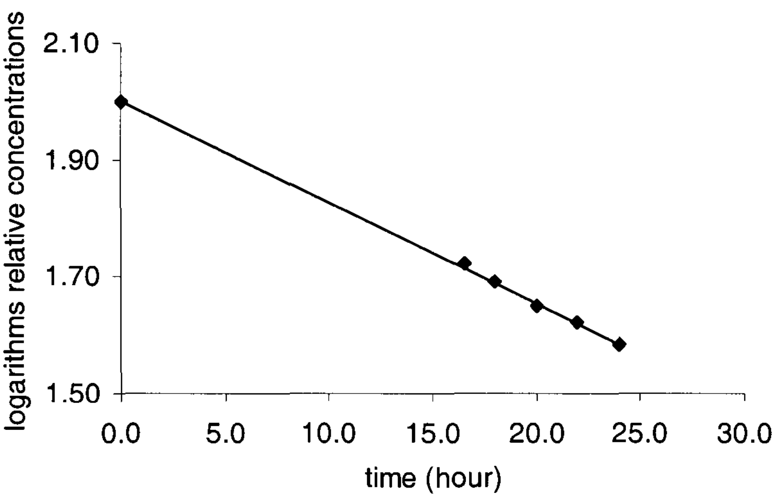

Figure 15 Plot of the logarithms of all relative concentrations against time for pH 9 at 39°C for MIPKP-T3 based on peak 1.

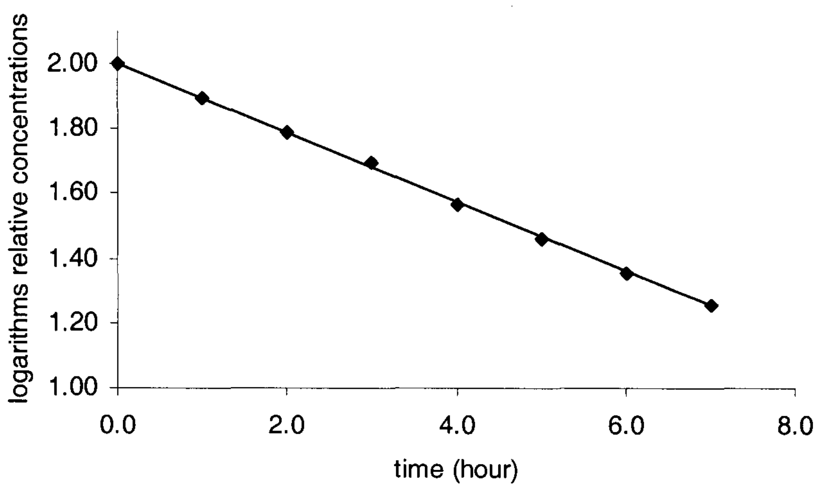

Figure 16 Plot of the logarithms of all relative concentrations against time for pH 9 at 50°C for MIPKP-T3 based on peak 2.

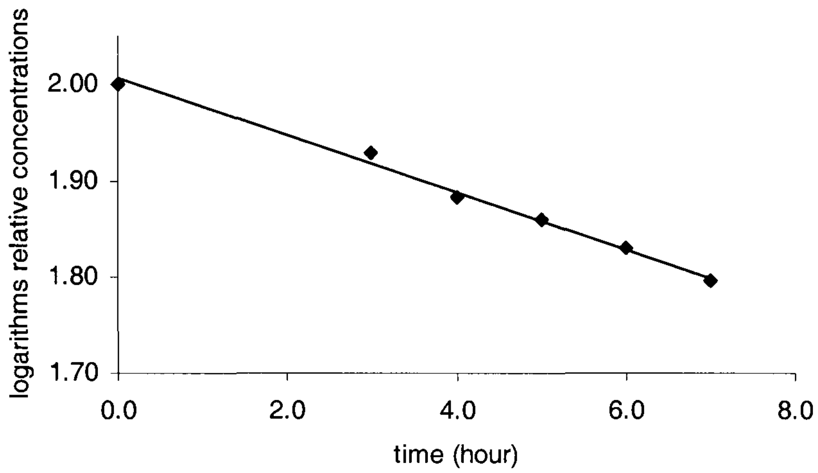

Figure 17 Plot of the logarithms of all relative concentrations against time for pH 9 at 39°C for MIPKP-T3 based on peak 2.

[redacted]

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis

[redacted]

[redacted]

ICS-331

Product name : [redacted]

[redacted]

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.11, Jo/95.2	Peroxidic compounds (sum) ***See page 2 for a specification***	% m/m	28.6 (± 1.5)
J20010792	Dimethyl phthalate [redacted]	% m/m	67.0 (± 1.0)
[redacted]			
[redacted]			
[redacted]		% m/m	0.5 (± 0.2)

*1 bracketed values are estimated 95% confidence intervals

File code : [redacted]

Analytical documentation : 20010792

[redacted]

[redacted]

[redacted]

Certificate of Analysis

[redacted]

[redacted]
page 2 of 2

[redacted] specification of the peroxidic compounds

structure	% m/m
[redacted]	[redacted]

[redacted]